drug resistance and the need to find novel classes of antimicrobial peptides as alternatives to antibiotics. Lactophoricin (LPcin), a cationic amphipathic peptide consists of 23-mer peptide, corresponds to the carboxy terminal 113-135 region of component-3 of proteose-peptone. LPcin is a good candidate as a peptide antibiotic because it has an antibacterial activity but no hemolytic activity. Three different analogs of LPcin, LPcin-yk2 which has mutant amino acids, LPcin-yk1 and LPcin-yk3 that has shorter mutant amino acids are recently developed by using peptide engineering in our laboratory. These three LPcin analogs show better antibioitic activities than wild-type LPcin and no toxicity at all. In order to understand the structural correlation between LPcin analogs structure and antimicrobial activity under the membrane environments, we tried to express and purify as large as amounts of LPcin and three different LPcin analogs. We finally optimized and succeed to overexpress in the form of fusion protein in *Escherichia coli* and purified with biophysical techniques like Ni-affinity chromatography, dialysis, entrifuge, chemical cleavage, and reversed-phase semiprep HPLC. In here, we will present the optimizing processes for high-yield expression and purification and solution NMR spectra and solid state NMR spectra for antimicrobial mechanisms.

974-Pos Board B729

NMR Analyses of the Structure and Dynamics of Klebsiella Pneumoniae OMPA Domains and Full Length Protein

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The transmembrane domain of kpOmpA possesses four long extracellular loops which exhibit substantial sequence variability throughout OmpA homologues in *Enterobacteria*. These loops are responsible for the immunological properties of the protein, such as cellular and humoral recognitions. Using liquid state NMR we have determined the 3D structure of kpOmpA in DHPC micelles (M. Renault et al., *J. Mol. Biol.* 2009). In a micellar environment, a complex dynamical behavior has been observed: a rigid barrel core, ms motion at the micellar-water interface, and sub-ns motion within the loops. Using solid state NMR relaxation and proteolysis experiments, we have demonstrated the persistence of this complex motional behavior in *E. coli* polar lipid bilayers (I. Iordanov et al., Biochim. Biophys. Acta, 2012). Using single molecule force spectroscopy (with D. Muller and A. Engel) we have shown that kpOmpA is able to unfold and refold reversibly its β-barrel core (P. Bosshart et al., *Structure* 2012).

Recent advances involve: a) characterizing the structure of its C-terminal domain and its interaction with the peptido-glycane; b) analyzing ssNMR spectra of N-terminal membrane domain in liposomes using MAS at 1 GHz and 60 kHz spinning frequency (with G. Pintacuda); c) comparing the NMR spectra of the various domains and the full length protein in solution, in liposomes and in intact cell envelopes using cellular solid state NMR as established in (M. Renault et al., PNAS 2012).

975-Pos Board B730

High-Resolution NMR Spectroscopy Reveals Structure of Lipoprotein flpp3

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Characterization of membrane proteins continues to be an enormous challenge in the field of structural biology compared to their soluble counterparts. Membrane proteins require the presence of a detergent micelle to protect the hydrophobic-exposed residues that are, under native conditions, inserted into a membrane. This provides challenging hurdles for expression, purification, and structural determination. However, for some lipoproteins and in the case of flpp3 from Francisella tularensis, the hydrophilic domain of the protein can be expressed and purified separately from the transmembrane domain. Flpp3 contains a single transmembrane helix at the N-terminus and therefore this technique allows for structural investigation of the majority of the protein without the necessity of a detergent micelle. In this study we report the results of expression, purification, biophysical characterization and NMR analysis of the soluble domain of flpp3 (flpp3-Sol). Sufficient quantities of pure flpp3-Sol are obtained via recombinant expression in *E. coli* cells followed by purification via Ni-NTA chromatography and gel filtration chromatography. Monodispersity and global secondary structure is investigated by dynamic light scattering (DLS) and circular dichroism (CD) respectively. NMR studies report local secondary structure via secondary chemical shifts and are used to generate a structure-model using CS-ROSETTA. Structures generated from NMR data are compared to structures in the Protein Data Bank using the Dali server and provide insight into possible protein function. The structure of flpp3-Sol is similar to the C-terminal domain of the lipoprotein bamC from *E coli*, a protein important for the insertion of beta-barrel proteins into the outer membrane of cells via the BAM complex; and as of yet unidentified in *F tularensis*.

Advances in Single-Molecule Spectroscopy I

976-Pos Board B731

A Sequential Monte Carlo Method for Identifying Motion Parameters from Particle Tracking Trajectories

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Optimal estimation of diffusion coefficients from single- and multiple-particle tracking data has undergone several breakthroughs in the past few years. Prior to these breakthroughs, the most common method for extracting motion parameters from trajectories was by fitting a curve to the mean square displacement [1]; it has been known for quite some time, however, that this a possibly inaccurate and unreliable method due to the presence of noise in the trajectory [2]. As a result, recent efforts by A. J. Berglund and X. Michalet have provided an optimal framework for trajectories corrupted by motion blur and Gaussian white noise [3]. These methods typically require that localization be performed prior to the estimation of motion parameters rather than in conjunction. These two problems, however, are dependent on each other. In this work, we present a Sequential Monte Carlo approach that utilizes the Expectation Maximization algorithm to simultaneously localize particle positions and estimate motion parameters from raw data (e.g. image sequences). The method provides a clear accuracy-vs-complexity trade-off and can be parallelized for greater efficiency. We demonstrate its effectiveness by detailing its use on arbitrary point spread functions, such as the double helix, and motion models, such as those driven by Markov jump processes.

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977-Pos Board B732

A Highly Specific Gold Nanoprobe for Live-Cell Single-Molecule Imaging in Confined Environments: Intracellular Tracking and Long-Term Single Integrin Tracking in Adhesion Sites

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Single molecule tracking in live cells is the ultimate tool to study subcellular protein dynamics, but it is often limited by the probe size and photostability. Due to these issues, long-term tracking of proteins in confined and crowded environments, such as adhesion sites, synaptic clefts or intracellular spaces, remains challenging. We present a novel optical probe consisting of 5-nm gold nanoparticles functionalized with a small fragment of camelid antibodies that recognize widely used GFPs with a very high affinity (1). These small gold nanoparticles can be detected and tracked using photothermal imaging for arbitrarily long periods of time (1-2). Surface and intracellular GFP-proteins can effectively be labeled even in very crowded environments such as adhesion sites and cytoskeletal structures both in vitro and in live cell cultures. Comparison with performances obtained by superresolution methods such as PALM and STED are presented for single integrin tracking in and out adhesion sites (3). These nanobody-coated gold nanoparticles are single molecule probes with unparalleled capabilities; small size, perfect photostability, high specificity, and versatility afforded by combination with the vast existing library of GFP-tagged proteins.

References:

(1) C. Leduc, et al Nano Lett. 13, 4, (2013) 1489.